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16. ABSTRACT

The main objective of genetic monitoring in human health surveillance is to prevent excessive exposure to genotoxic agents and to ensure the safety of the exposed or potentially-exposed populace. To attain fully this objective one must understand low dose exposure and associated effects so as to relate both to long-term health consequences. Experience with radiation has provided the most precise knowledge of the dose-effect relationlship, and it is that knowledge that we wish to exploit in the present investigation. Over the past few years our laboratory has implemented and further developed several methods for cytogenetic and mutational testing and monitoring. These methods employ periphral blood lymphocytes and fibroblasts to detect chromosomal structural aberrations, sister-chromatid exchanges, and gene mutation at specific genetic loci. Parallel methods have been established using human and rodent cell systems both in vitro and in vivo in an attempt to correlate laboratory derived dose-response data with those obtained using cells of individuals exposed in clinical and occupational settings. Our studies to date have dealt with λ-radiation, ethylene oxide, cyclophosphamide, benzo(a) pyrene and ethyl and vinyl carbamate.

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A PRELIMINARY INVESTIGATION OF THE PARALLELOGRAM CONCEPT IN GENETIC MONITORING AND RISK ESTIMATION

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INTRODUCTION

The principal objective in monitoring humans for occupational exposure to DNA-damaging agents is to ensure the health and safety of the exposed working population. Ideally, the monitoring of subtle genotoxic effects of low levels of exposure could be used in estimating the risk of cancer or genetic disease. However, if the genetic damage is repaired, little or no evidence may exist that can be related to heritable mutations or tumors in the exposed population. Studies using low chronic exposures are also limited because they may not produce a measurable genotoxic effect or evidence of an increased disease frequency. Thus, within the limits of current methodology, some combination of animal and short-term genetic tests must be used to supplement available human data. Data derived from experimental systems cannot be used to predict human health risk without the development of appropriate quantitative extrapolation methods. We have recently begun the process of evaluating such a quantitative method based upon the application of available bioassay techniques (Waters et al., 1984). The origins of this work may be found in biological approaches to radiation dosimetry and in the parallelogram concept described originally by Sobels (1977, 1982).

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S.EPA and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

The parallelogram method is an approach involving somatic and/or germ cell monitoring techniques whereby quantitative dose-response relations are developed among component test systems. In its simplest form, this approach (illustrated in Fig. 1) attempts to relate mathematically the potency of a test agent in (rodent or human) cells exposed *in vitro* to the potency measured in the same type cells exposed *in vivo*. These relations are expressed as constants a and c in Fig. 1. The *in vitro* and *in vivo* responses in the two species are in turn related as constants b and d, respectively. If the relations are parallel, then a = c and b = d. Once the constants have been determined for comparable agents and effects, it may then be possible to extrapolate in situations where human *in vivo* data are unavailable.

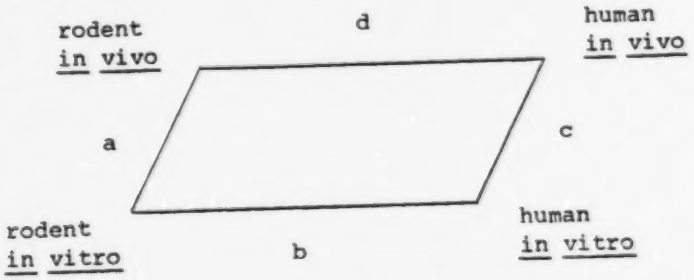

Figure 1. Parallelogram method for extrapolation.

In practice, one determines either the dose to the DNA (target dose) or the dose causing the modification of a nucleotide or nucleotide sequence (molecular dose) to provide the quantitative basis for extrapolating data from animal systems to human populations. The relative potency of individual agents may be subject to further quantitation if the kinds of DNA adducts formed and their distribution within the molecule after exposure can be characterized in the various test systems and tissues involved in the parallelogram. Data exist in the literature (Singer, 1979) that indicate that if the molecular target is closely related to the genetically significant target, the number of reaction products (adducts) formed may be proportional to the number of genetic alterations. The reaction products measured may not be those responsible for genetic change, although, if a proportionality holds, the relative potency of an agent can be determined.

In performing the preliminary assessment described in this report, we have emphasized the use of agents for which human

data are available. Studies with ionizing radiation have provided the most precise knowledge of the dose-effect relations in humans. Thus, we have selected gamma radiation based upon the extensive data on chromosome aberrations induced by this agent in human peripheral blood lymphocytes (PBLs). The human PBL cytogenetics system in vitro has been successfully employed as a biological dosimeter (Lloyd et al., 1975, 1984; Littlefield et al., 1980; Sasaki, 1983) to estimate absorbed gamma radiation dose in humans. We have attempted to develop a similar model based on the use of PBLs from the mouse. The parallelogram illustrated in Fig. 1 has been investigated using mouse and human PBLs exposed to ^{137}Cesium gamma radiation. The potential utility of the data from the mouse model together with the data from the human in vitro system has been examined relative to cytogenetic alterations reported by Buckton et al. (1971) in exposed humans.

Although ionizing radiation and most chemical genotoxic agents have different molecular mechanisms of interaction with DNA, it may be possible to utilize a similar approach in studies with chemicals. Chemotherapeutic drugs used at high dose levels in carefully controlled clinical settings are ideal genotoxic agents for such investigations. Therefore, the second agent selected for study was the cytostatic drug cyclophosphamide (CP). CP is a nitrogen mustard compound widely used in cancer chemotherapy; it induces a myriad of genetic effects in a wide variety of bioassay systems (Waters et al., 1983). The induction of sister chromatid exchanges (SCE) by CP has been studied in mouse PBLs in vitro and in vivo (Wilmer et al., 1984). Both CP and the principle active metabolite, phosphoramide mustard (PAM), have been evaluated for SCE induction in human PBLs in vitro (Wilmer et al., in press). Thus, a comparison may be drawn between results obtained with CP and with PAM in human PBLs in vitro, and both may be compared with the published in vitro data. Also, some published data are available to construct a dose-response curve for induction of SCE in humans treated therapeutically with CP (Clare et al., 1982). We do not have DNA adduct dosimetry for the experiments described on CP; however, the PBL data cited should facilitate future dosimetric studies.

The third agent selected for investigation was benzo(a)-pyrene [B(a)P], a widely-dispersed suspect human carcinogen. A substantial amount of in vitro data has been developed in our laboratory measuring B(a)P-induced genotoxic effects and DNA adducts in several short-term bioassay systems including SCE in Chinese hamster V79 cells (Nesnow et al., 1985). In

the present investigation, we have added experiments on the induction of SCE in mouse PBLs *in vivo*. Both total B(a)P-DNA binding and specific B(a)P-DNA adducts were measured. A modification of the doubling-dose concept taken from radiation biology was applied to the DNA adduct and genetic toxicology data (cf. Fig. 2). This modification, the doubling adduct level, is defined as the number of DNA adducts per unit of DNA, required to double the induced frequency of genotoxic response. This approach (given certain limitations) allows comparisons of potencies of chemicals to be made where the dose or concentration administered is converted to internal dose to the DNA irrespective of the absolute frequencies of the events.

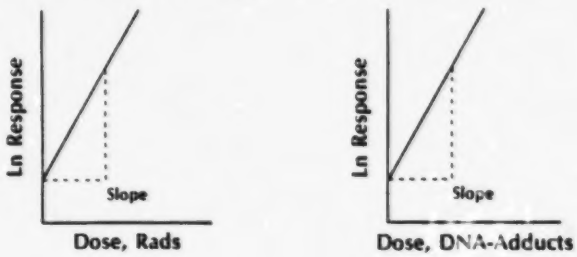

Figure 2. Comparison of doubling dose and doubling adduct level.

$$\text{Doubling Dose} = \frac{0.693}{\text{Slope}} \qquad \text{Doubling Adduct Level} = \frac{0.693}{\text{Slope}}$$

The studies described below represent our initial efforts to implement and evaluate the parallelogram concept using cytogenetic and dosimetric data. The results are incomplete but define our progress to date in attempts to determine the validity of the parallelogram concept.

METHODS

Gamma ray exposures were performed using a ^{137}Cesium dual-source irradiator (J.L. Shepard, model 431) providing an unattenuated dose rate of 2100 rad/min. Low doses were delivered as required through lead attenuators. A calibrated X-ray monitor was employed to measure directly cumulative delivered doses.

Male, C57BL/6 mice, 3-5 months old, (Jackson Laboratory) were used for all mouse PBL studies. *In vivo* and *in vitro* chemical exposures and all cell culture and cytogenetic methods followed our usual procedures (Wilmer et al., 1984; Kligerman et al., in press). For human PBLs, blood was drawn from a 29-year old male and cultured according to the methods described previously by Allen et al. (1984). Chromosome interchanges were

analyzed from first division metaphases ascertained with BrdUrd labeling after treatment. DNA adducts in mouse PBLs were determined by R. C. Gupta (Baylor College of Medicine) using a ^{32}P-postlabeling method (Randerath et al., 1981).

Quadratic regression models were fit to all cytogenetic aberration dose-response data using the method of least squares. For data on dicentrics/cell, these models were forced through zero at dose zero.

RESULTS AND DISCUSSION

Gamma radiation

Two important physical aspects of radiation exposure of human PBLs pertain to radiation quality and delivery. Radiation quality can be described in terms of linear energy transfer (LET). In general, high-LET radiation (e.g., neutrons, alpha particles) results in linear dose-response curves. The linear dose-response model is $Y = \alpha D$, where Y is aberration yield (in human PBL cytogenetics usually described by dicentrics per cell), α is the slope coefficient of the curve, and D is the dose in rads. With low-LET radiation (e.g., gamma rays), the $Y = \alpha D$ model requires an additional term such that the quadratic equation, $Y = \alpha D + \beta D^2$, is necessary to adequately describe the relation. In terms of cytogenetics, the explanation for the quadratic dose-response curve is that some dicentrics are caused by single energy deposition events (hits), while others are caused by the interaction of two events (thus, increasing with the square of the dose). The α and β coefficients have been derived by numerous authors for X-rays and ^{60}Cobalt gamma rays (see Lloyd et al., 1975) and are in general agreement.

The other major physical factor involved in cytogenetic dose-response determinations is the dose rate, which is important for low-LET radiation. In the quadratic equation, the βD^2 component is dependent on dose rate because it is presumed to result from an interaction of events and is thus lower when the events are separated further in time. When the dose rate for low-LET radiation decreases to near environmental levels, the βD^2 term may be eliminated as was demonstrated by Evans et al. (1979). For a discussion on the prediction of dicentric yields produced in lymphocytes chronically exposed to gamma rays, see Lloyd et al. (1984).

To our knowledge, there are only two reports describing the induction of cytogenetic effects in human PBLs in vitro by ^{137}Cesium (Brewen and Luippold, 1971; Doggett and McKenzie, 1983). Data points (Fig. 3) resulting from our limited investigation and those reported by Doggett and McKenzie (1983) were examined and determined not to differ statistically. Thus, it was possible to pool the data for the construction of the dose-response curve and estimation of the α and β coefficients according to the equation $Y = \alpha D + \beta D^2$. The estimated α and β values for human PBLs in vitro were 17.2×10^{-4} aberrations/rad and 2.42×10^{-6} aberrations/rad^2, respectively.

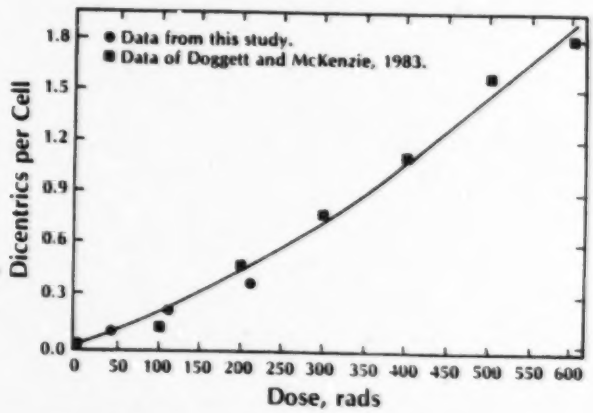

Figure 3. Effect of ^{137}Cesium irradiation on human PBLs in vitro.

Using coefficients similarly determined from our preliminary studies on rodent PBLs and derived from the literature (Buckton et al., 1971) for human PBLs in vivo, it was possible to predict dicentrics/cell at 25, 50, 100, and 200 rad doses for each component of the parallelogram (Table 1).

Table 1. **Predicted Values for Dicentric Aberrations/Cell Based upon the Equation $Y = \alpha D + \beta D^2$**

Test	Dose			
	25 Rads	50 Rads	100 Rads	200 Rads
Mouse PBLs *in vitro*	0.005	0.011	0.030	0.091
Mouse PBLs *in vivo*	0.003	0.008	0.027	0.093
Human PBLs *in vitro*	0.044	0.092	0.196	0.440
Human PBLs *in vivo*	0.020	0.048	0.126	0.371

The extrapolation constants derived from the predicted dicentrics/cell varied with dose but appeared to be similar for the corresponding sides of the parallelogram (Fig. 4). While these results are consistent with the parallelogram

extrapolation concept, they are nonetheless preliminary. It would be premature to use these data to judge the validity of the concept.

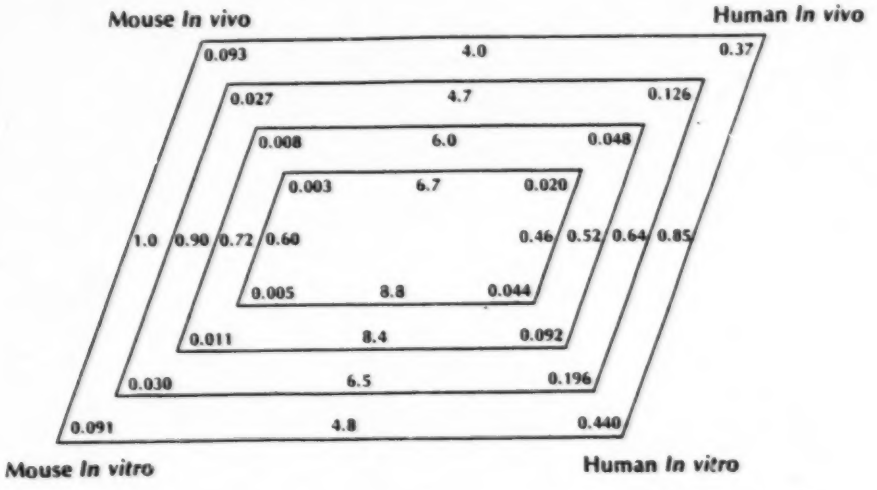

Figure 4. Preliminary evaluation of the parallelogram model using predicted values of dicentrics/cell (from the innermost parallelogram, the doses represented are 25, 50, 100 and 200 rads, respectively).

Cyclophosphamide (CP)

To date we have completed only a feasibility study on the CP parallelogram based on an examination of the available SCE data (Table 2). Wilmer et al. (1984) determined the SCE-inducing potential of CP in mouse T-lymphocytes after both in vitro and in vivo exposure. CP was added to whole blood mouse PBL cultures 24 h after stimulation with phytohemagglutinin (PHA), and the cells were harvested 36 h later. At concentrations from 0.05 to 0.5 mM CP, there was little increase in SCE frequency. However, from 0.8 to 1 mM a twofold increase in SCE frequency over baseline was apparent. This is similar to the response seen in rat PBLs (Waalkens et al., 1981).

Wilmer et al. (in press) have also shown that human PBLs stimulated with concanavalin A and exposed to CP for the final 48 h of culture can activate CP (Table 2). In this system 1 mM CP led to an approximately twofold increase over

Table 2. SCE Responses in Lymphocytes

Species	Chemical	Effective Dose Range	Dose that Gives Twofold over Background	Reference
Mouse In vitro	CP w/o S-9	0.5 - 1.0 mM	0.8 - 1 mM	Wilmer et al., 1984
Rat In vitro	CP w/o S-9	0.1 - 2.0 mM	0.5 - 1 mM	Waalkens et al., 1981
Human In vitro	CP w/o S-9	0.5 - 2.0 mM	1 mM	Wilmer et al., in press
Human In vitro	CP w/o S-9	0.5 - 2.0 mM	1 mM	Waalkens et al., 1981
Human In vitro	CP w/o S-9	0.5 - 2.0 mM	1 mM	Norppa et al., 1983
Human In vitro	CP w/ S-9	7 nM- 8.7µM	0.01-0.1 µM	Clare et al., 1982
Human In vitro	PAM w/o S-9	0.0068- 3.4 µM	0.1 µM	Wilmer et al., in press
Mouse In vivo	CP	1.0-5.0 mg/kg	5 - 6 mg/kg	Wilmer et al., 1984
Rat In vivo	CP	13.3 mg/kg	4 - 5 mg/kg	Basler 1982
Human In vivo	CP	14.9 mg/kg	3 - 4 mg/kg	Clare et al., 1982

baseline SCE frequency (Table 2), as in the findings of Waalkens et al. (1981) and Norppa et al. (1983). However, PAM, the major genotoxic metabolite of CP, caused a doubling in SCE frequency at 0.1 μM. Thus, apparently the PBLs were only able to activate approximately 0.01% of the CP. In concordance with these results, Clare et al. (1982), showed that 0.01 to 0.1 μM CP induced a doubling of SCE over baseline in whole blood human PBL cultures in the presence of rat liver S-9.

In the mouse *in vivo* study of Wilmer et al., 1984 (Table 2), CP was administered ip, and blood was drawn and cultured 24 h after exposure. A statistically significant increase in SCE frequency was found at 1 mg/kg, and by linear extrapolation the dose that caused a twofold increase in SCE should be between 5 and 6 mg/kg. Similarly, we have calculated from the published data of Basler (1982) for rat PBLs (following *in vivo* exposure to 13.3 mg CP/ kg) that the dose leading to a twofold increase in SCE over baseline should be between 4 and 5 mg/kg.

Although the literature on human exposure to CP *in vivo* is substantial, it is difficult to make any realistic dose-response estimates from the data because of variable or uncertain information concerning the exposure regimens, sampling times, routes of administration, and culture conditions used. In order to make comparisons between the human and the rodent data, it was necessary to select studies in which CP was administered only once, and the blood was sampled and cultured within one week of the exposure. Clare et al. (1982) found that blood withdrawn from a patient within 22 h after iv administration of 14.9 mg/kg showed over a sixfold increase in SCE. By linear interpolation, one could estimate the dose that would cause a twofold increase over baseline to be between 3 and 4 mg/kg. Again, this is close to the values found for the mouse (Wilmer et al., 1984) and the rat (Basler, 1982). Szollar et al. (1983) found that one day following administration of 600 mg CP/m^2 to children with tumors, there was an increase of about 12 SCEs over baseline. By converting the dose to mg CP/m^2 (Freireich et al., 1966) and interpolation, one can estimate the dose that leads to a twofold increase over baseline to be about 11 mg/kg. Despite the uncertainty involved in this estimation (i.e., age, weight, sex not reported), these data are in agreement with those reported in the animal studies. Thus, our review of the SCE data in the literature suggests that CP will be a useful agent for further study of the parallelogram model.

Benzo(a)pyrene [B(a)P]

While the metabolism of CP has been studied for some time, there are limited data on its adducts to DNA, especially with *in vivo* systems. It is known, however, that a DNA adduct of PAM identified to date is an unstable N-7 adduct to guanine, which rapidly undergoes ring scission (Mehta et al., 1980; Chetsanga et al., 1982). Other adducts also have been identified including ones involving crosslinking of the mustard (Vu et al., 1981). These adducts are unstable *in vitro*, and their stability *in vivo* has not yet been reported, although preliminary studies indicate that they may be more stable *in vivo* (Kallama and Hemminki, 1984). Thus, DNA adduct dosimetry with CP may be difficult and may require new methodology.

Genotoxic effects have been demonstrated with B(a)P in both rodent and human tissues. It is a suspect human carcinogen and its metabolism, metabolic activation, and DNA adducts have been thoroughly studied (International Agency for Research on Cancer, 1983; Singer and Grunberger, 1983). Nesnow et al. (1985) has reported on the doubling adduct levels of B(a)P for SCE in Chinese hamster V79 cells using the B(a)P-DNA adduct, N^2-(10β[7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene]yl)-deoxyguanosine [BPDE I-dG]. We have derived similar data on SCE formation in PBLs from C57BL/6 mice. Thus, a comparison can be made between doubling adduct levels between mouse PBLs and V79 cells in order to estimate the extrapolation constant for SCE between rodent tissues *in vitro* and *in vivo*. This constant is 0.37 based on the ratio of 0.19 and 0.50 for the doubling adduct levels of mouse PBL and V79 cells, respectively. The assumption implicit in the use of the doubling adduct level is that the relation between the frequency of genetic response and the DNA adduct level is an exponential one. In the data presented here the complete dose response has not been elucidated and an exponential relation has been assumed. These data do not as yet permit a complete evaluation of doubling adduct levels with the B(a)P parallelogram, but no future difficulties are expected since the adducts are well defined and quite stable.

CONCLUSIONS

Data have been presented on three agents that will be of use in evaluating the applicability of the parallelogram concept in genetic monitoring and risk estimation. The biological system selected, cytogenetic alterations in PBLs, offers one of the simplest and potentially most useful models for occupational

and environmental monitoring. If the parallelogram concept can be validated using the PBL model, it should be possible to generate useful experimental data on various kinds of genetic and molecular effects in rodents and in human cells *in vitro*. Hopefully, these data will be directly applicable in assessing the potential impact of genetic alterations detected in human PBLs *in vivo*. In any case, knowledge of the utility of the PBL system in genetic health surveillance will be enhanced.

REFERENCES

Allen JW, Brock K, Campbell J, Sharief Y (1984). Sister chromatid exchange analysis in lymphocytes. In Ansari AA, deSerres FJ (eds): "Single-Cell Mutation Monitoring Systems: Methodologies and Applications," New York: Plenum Press, pp 145-163.

Basler A (1982). SCEs in lymphocytes of rats after exposure *in vitro* to cigarette smoke or to cyclophosphamide. Mutation Res 102:137-143.

Brewen JG, Luippold HE (1971). Radiation-induced human chromosome aberrations: *In vitro* dose rate studies. Mutation Res 12:305-314.

Buckton KE, Langlands AO, Smith PG, Woodcock GE, Looby PC (1971). Further studies on chromosome aberration production after whole-body irradiation in man. Int J Radiat Biol 19:4:369-378.

Chetsanga CJ, Polidori G, Mainwaring M (1982). Analysis and excision of ring-opened phosphoramide mustard-deoxyguanosine adduct in DNA. Cancer Res 42:2616-2621.

Clare MG, Jones WG, Taylor JH (1982). Sister chromatid exchanges in human lymphocytes exposed to single cytotoxic drugs *in vivo* or *in vitro*. Eur J Cancer Clin Oncol 18:10: 979-989.

Doggett NA, McKenzie WH (1983). An analysis of the distribution and dose response of chromosome aberrations in human lymphocytes after *in vitro* exposure to ^{137}Cesium gamma radiation. Radiat Environ Biophys 22:33-51.

Evans HJ, Buckton KE, Hamilton GE, Carothers A (1979). Radiation-induced chromosome aberrations in nuclear-dockyard workers. Nature 277:531-534.

Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966). Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemotherapy Report 50:4:219-244.

International Agency for Research on Cancer (1983). "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol 32 Polynuclear Aromatic Compounds Part I. Chemical, Environmental and Experimental Data," Lyon: IARC, pp 211-224.

Kallama S, Hemminki K (1984). Alkylation of guanosine by phosphoramide mustard, chloromethine hydrochloride and chlorambucil. Acta pharmacol et toxicol 54:214-220.

Kligerman AD, Erexson GL, Westbrook-Collins B, Allen JW (In press). Analysis of sister chromatid exchange in mouse peripheral blood lymphocytes following in vivo exposure to carcinogen/noncarcinogen pairs. In Ashby J: de Serres FJ, Shelby MD, Margolin B, Ishidate M. Jr., Becking, GC (eds): "Evaluation of Short-Term Tests for Carcinogens", Amsterdam: Elsevier Science Pub.

Littlefield LG, Joiner EE, DuFrain RJ, Hubner KF, Beck WL (1980). Cytogenetic dose estimates from in vivo samples from persons involved in real or suspected radiation exposures. In Hubner KF, Fry SA (eds): "The Medical Basis for Radiation Accident Preparedness," New York: Elsevier/North-Holland, pp 375-390.

Lloyd DC, Edwards AA, Prosser JS, Corp MJ (1984). The dose response relationship obtained at constant irradiation times for the induction of chromosome aberrations in human lymphocytes by cobalt-60 gamma rays. Radiat Environ Biophys 23:179-189.

Lloyd DC, Purrott RJ, Dolphin GW, Bolton D, Edwards AA (1975). The relationship between chromosome aberrations and low LET radiation dose to human lymphocytes. Int J Radiat Biol 28:1:75-90.

Mehta JR, Przybylski M, Ludlum DB (1980). Alkylation of guanosine and deoxyguanosine by phosphoramide mustard. Cancer Res 40:4183-4186.

Nesnow S, Moore MM, Allen JW, Grunberger D, Theall-Arce G (1985). The use of the doubling dose concept with DNA adduct dosimetry in the evaluation of short-term bioassays. Proc Amer Assoc Cancer Res 26:96.

Norppa, H, Vainio H, Sorsa, M (1983). Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. Cancer Res 43:3579-3582.

Randerath K, Reddy MV, Gupta RC (1981). ^{32}P-labeling test for DNA damage. Proc Natl Acad Sci (USA) 78:6126-6129.

Sasaki MS (1983). Use of lymphocyte chromosome aberrations in biological dosimetry: Possibilities and limitations. In Ishihara T, Sasaki MS (eds): "Radiation-Induced Chromosome Damage in Man," New York: Alan R Liss, pp 585-604.

Singer B (1979). N-nitroso alkylating agents: Formation and persistance of alkyl derivatives in mammalian nucleic acid as contributing factors in carcinogenesis, J Natl Cancer Inst, 62:1329-1339.

Singer B, Grunberger D (1983). "Molecular Biology of Mutagens and Carcinogens," New York: Plenum Press, 347 pp.

Sobels FH (1977). Extrapolation from experimental test systems for evaluation of genetic risks in man (Chairman's Remarks on Panel Discussion). In Scott D, Bridges BA, Sobels FH (eds): "Progress in Genetic Toxicology," New York: Elsevier/North-Holland, pp 175-182.

Sobels FH (1982). The parallelogram: An indirect approach for the assessment of genetic risks from chemical mutagens. Prog Mutat Res 3:323-327.

Szollar J, Fekete G, Schuler D (1983). Sister chromatid exchange in childhood malignancies. Teratogenesis, Carcinogenesis, and Mutagenesis 3:527-533.

Vu VT, Fenselau CC, Colvin OM (1981). Identification of three alkylated nucleotide adducts from the reaction of guanosine-5'-monophosphate with phosphoramide mustard. J Amer Chem Soc 103:7362-7364.

Waalkens DH, Joosten HFP, Taalman RDFM, Scheres JMJC, Yih TD, Hoekstra A (1981). Sister-chromatid exchanges induced in vitro by cyclophosphamide without exogenous metabolic activation in lymphocytes from three mammalian species. Toxicology Letters 7:229-232.

Waters MD, Garrett NE, Covone-de Serres CM, Howard BE, Stack HF (1983). Genetic toxicology of some known or suspected human carcinogens. In de Serres FJ (ed): "Chemical Mutagens: Principles and Methods for Their Detection," Vol 8, New York: Plenum Press, pp 261-341.

Waters MD, Allen JW, Claxton LD, Garrett NE, Huang SL, Moore MM, Sharief Y, Strauss GH (1984). Evaluation of genotoxic effects in human populations. In de Serres FJ, Pero RW (eds): "Individual Susceptibility to Genotoxic Agents in the Human Population," New York: Plenum Press, pp 53-87.

Wilmer JL, Erexson GL, Kligerman AD (1984). Sister chromatid exchange induction in mouse B- and T-lymphocytes exposed to cyclophosphamide in vitro and in vivo. Cancer Res 44: 880-884.

Wilmer JL, Erexson GL, Kligerman, AD (In press). Attenuation of cytogenetic damage by 2-mercaptoethanesulfonate in cultured human lymphocytes to exposed to cyclophosphamide and its reactive metabolites. Cancer Res.